

### **Remarks**

We note with appreciation the Examiner's detailed and extensive helpful comments with respect to the drawings, sequence rules, claim of priority, specification and many of the claims. We have made amendments/changes in accordance with those helpful changes to place the entire application into condition for allowance.

We have amended Fig. 2 in accordance with the Examiner's helpful suggestion with respect to identification with the corresponding SEQ ID NOs.

We have amended the specification in accordance with the Examiner's helpful suggestions, especially with respect to page 22, line 1 and the Brief Description of the Drawings. We have made a number of additional miscellaneous changes as to form and idiom in the specification to place it into better form under U.S. Rules of Practice and to improve readability. We enclose herewith a substitute specification for the Examiner's convenience, together with a marked-up copy showing the changes. No new matter has been added.

We have also amended all of the claims to place them into final condition for allowance. A number of the changes are minor in nature and are simply addressed to format, idiom and/or readability. Others of the changes have been made directly in accordance with the Examiner's helpful suggestions for clarification purposes.

Starting at Claim 1, it has been amended in accordance with the Examiner's helpful suggestion to recite an isolated and purified protein. It has further been amended to cite a mammalian neuronal ASIC cationic channel. Support may, of course, be found throughout the specification. We respectfully submit that Claim 1 is in compliance with §101 with respect to statutory subject matter and further provides structural limitation on the claims such that the meets and bounds of the claims can easily be determined, especially in light of the characterization as being "ASIC".

Claims 2, 3, 5, 11, 12, 13, 15, 17, 18, 19, 21, 24 and 27 have been amended to correct minor matters of form or idiom. These changes are simply made to place those claims into better condition for allowance.

We note that Claim 11 was not amended along with Claim 1 with respect to providing a structural limitation, as discussed above with respect to Claim 1, inasmuch as Claim 11 depends from Claim 1 and contains the same subject matter.

Claims 2, 3 and 5 were also not amended to further clarify the meaning of “functionally equivalent derivative.” In that regard, we respectfully submit that this term is clearly definite in view of the extended discussion defining its meaning in the Applicants’ specification. We particularly invite the Examiner’s attention to page 2 of the specification wherein a definition of this term is provided. The last two lines of page 1 refer to the functionally equivalent derivative of the protein. The beginning of page 2 defines what is meant by such derivatives. This is explicitly set forth in the first paragraph of page 2. Moreover, the explanation/definition of the derivatives does not stop with a word definition, but proceeds in the second and third paragraphs and extending through page 3, specific examples of what are included within the meaning of “functionally equivalent derivatives”. As an example, ASIC1A is included as is MDEG1 and ASIC2A. Explanations associated with each of these examples are provided. We accordingly respectfully submit that one of ordinary skill in the art would clearly understand what the Applicants mean by “functionally equivalent derivatives.”

Claim 17 has been amended in accordance with the Examiner’s helpful suggestion by removing “advantageously” which is superfluous.

Claims 20 and 26 have been amended to provide a proper Markush grouping and we respectfully submit that those claims are now in proper form.

Claims 22 and 28 have been amended in accordance with the Examiner's helpful suggestions to provide appropriate method steps in the method for screening. Claims 22 and 28 now recite a contacting step, a measuring step and a determination step. The determination step recites that there is a determination that the substance is capable of modulating activity of the mammalian neuronal cationic channels if the change in current is above a selected value.

Claims 23 and 29 have been amended in accordance with the Examiner's helpful suggestions. Applicants have not removed "the perception of" because it would change the meaning of those claims in an inaccurate way. Testing pain perception and taste perception can be done by standard techniques in animals and/or in humans well-known by one skilled in the art, as disclosed in several references cited hereafter. We respectfully submit that Claims 23 and 29 are now also in proper form.

Claim 24 has been amended to remove the non-elected reference to antibody and respectfully submit that it is now also in proper form for allowance.

In light of the above amendments to the claims, we respectfully request withdrawal of the 35 U.S.C. §112, 2<sup>nd</sup> paragraph rejections and respectfully request prompt allowance of those claims.

Turning now to the 35 U.S.C. §101 rejection based on utility, we respectfully submit that the solicited claims are in full compliance with §101. There is no doubt that the claimed invention has clear utility in accordance with §101. This is fully supported by the Applicants' specification, described below, and further fully supported by the enclosed materials, also described below.

First, we invite the Examiner's attention to the specification, starting at page 8. This paragraph is the beginning of an extended discussion of the utility of the invention which spans at least through the bottom of page 11. The specification makes it clear that cells expressing ASIC channels or channels exhibiting the properties and structure of the type of the ASIC channels are useful for the screening of substances capable of modulating the perception of acidity regarding both nociception and taste transduction. The screening procedure makes it possible to identify drugs useful in treatment or prevention of pain. They also enable investigation of agents that modulate acid taste. They further enable investigation of blockers that inhibit neurodegenerations induced by hyperexpression of the channels.

A protein constituting an ASIC neuronal ionic channel can also be used to fabricate drugs intended for the treatment or prevention of pathologies entailing the painful perception of acidity which intervenes in inflammatory diseases, ischemias and selected tumors. The invention, therefore, provides for pharmaceutical compositions comprising as an active ingredient at least one protein constituting an ASIC channel.

The specification further points out that isolated and purified nucleic acid molecules of the invention coding for a protein constituting an ASIC channel or vectors including the nucleic acid molecules or cells expressing the ASIC channel may be utilized for preparing transgenic animals. Such animals have a deficiency in the ASIC channel and may be used as live models for studying animal diseases associated with ASIC channels.

We respectfully submit that these aspects of the invention clearly demonstrate utility under §101 without a doubt. Nothing could be more useful under §101 than providing a means to treat a wide variety of serious diseases. This invention is a novel and unobvious advance in the art which can potentially bring about great benefit to the public. This is the essence of utility under §101. The enclosed articles mentioned above also confirm the utility

of the claimed invention. The ASIC1 knockout mice disclosed in Wemmie et al., 2002 (Neuron, vol. 34, pages 463-477) display defective spatial learning and eyeblink conditioning. These data prove that inactivation of ASIC1 affect learning.

Violley et al., 2001 (J. Neurosci., vol. 21, pages 8026-8033) disclose that ASIC subunits are up-regulated during the inflammation and that ASIC3 might be a target for nonsteroid anti-inflammatory drugs. Moreover, the inactivation of ASIC2 (BCN1) causes decreased mechanoperception, as disclosed in Price et al., 2000 (Nature, vol. 407, pages 1007-1011), while the inactivation of ASIC3 causes altered mechanosensation and acid-evoked nociception (Price et al., 2001, Neuron, vol. 32, pages 1071-1083). Chen et al., 2002 (PNAS, vol. 99, pages 8992-8997) also disclose that inactivation of ASIC3 causes altered nociception.

In addition, Sutherland et al., 2001 (PNAS, vol. 98, pages 711-716) suggest that ASIC3 might play an important role in the perception of pain during cardiac ischemia, while brain ASIC channel activity seems to be involved in ischemic damage (Allen and Attwell, 2002, J. Physiol., vol. 543, pages 521-529).

With respect to enablement under §112, we respectfully submit that the amendment to Claim 1 with respect to providing the structure associated with its identification as "ASIC" we respectfully submit that the specification is fully enabling of all of the solicited claims.

In light of the foregoing, we respectfully submit that the entire application is now in condition for allowance, which is respectfully requested.

Respectfully submitted,



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**In the Claims** (clean copy as amended)

*But E1*  
*D2* 1) (Amended) An isolated and purified protein constituting a mammalian neuronal cationic ASIC channel that is sensitive to amiloride and activated by protons.

2) (Twice Amended) The protein according to claim 1, the amino acid sequence of which is SEQ ID NO: 2 or a functionally equivalent derivative.

*D3* 3) (Twice Amended) The protein according to claim 1, the amino acid sequence of which is SEQ ID NO: 4 or a functionally equivalent derivative.

*D4* 5) (Twice Amended) The protein according to claim 1, the amino acid sequence of which is SEQ ID NO: 8 or a functionally equivalent derivative.

*D5*  
*But E1* 11) (Twice Amended) A nucleic acid molecule comprising a nucleic sequence coding for the protein constituting a cationic channel according to one of claims 1, 2, 3 and 5.

*D6* 12) (Amended) The nucleic acid molecule according to claim 11, comprising the nucleic sequence bounded by nucleotides 123 and 1700 of the sequence represented in SEQ ID NO: 1.

13) (Twice Amended) The nucleic acid molecule according to claim 11, comprising the nucleic sequence bounded by nucleotides 1 and 1542 of SEQ ID NO: 3.

*D7* 15) (Twice Amended) The nucleic acid molecule according to claim 11, comprising the nucleic sequence bounded by nucleotides 109 and 1785 of SEQ ID NO: 7.

*D8* 17) (Twice Amended) A vector comprising at least one nucleic acid molecule according to claim 11, combined with control sequences.

*But E1* 18) (Twice Amended) A method for producing a protein according to one of claims 1, 2, 3 and 5 comprising:

- transferring a nucleic acid molecule comprising a nucleic acid sequence encoding the protein or a vector comprising said nucleic acid molecule into a cell host,
- culturing said cell host under conditions allowing production of the protein, and
- isolating the protein.

19) (Twice Amended) A method for expressing a protein according to one of claims 1, 2, 3 and 5 comprising:

- transferring a nucleic acid molecule comprising a nucleic acid sequence encoding the protein or a vector comprising said nucleic acid molecule into a cell host, and
- culturing said cell host under conditions allowing production of the protein.

20) (Twice Amended) The method according to claim 18, wherein the cell host is a bacteria or a eukaryote cell selected from the group consisting of yeasts, mammal cells, plant cells and insect cells.

21) (Twice Amended) A transformed cell expressing the mammalian neuronal amiloride-sensitive proton-activated cationic channels obtained by the method according to claim 18.

22) (Twice Amended) A method for screening a substance capable of modulating activity of mammalian neuronal cationic channels, comprising:

contacting variable quantities of a substance to be tested with the cells according to claim 21;

measuring changes in current caused by the substance on amiloride-sensitive proton-activated cationic channels; and

determining that the substance is capable of modulating activity of the mammalian neuronal cationic channels if the change in current is above a selected value.

23) (Twice Amended) The method according to claim 22, wherein said substance is capable of modulating the perception of acidity and affect nociception and taste transduction.

24) (Twice Amended) A pharmaceutical composition comprising as active ingredient at least one protein according to one of claims 1, 2, 3 and 5.

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26) (Amended) The method according to claim 19, wherein the cell host is selected from the group consisting of prokaryotes of bacteria, yeasts or cells of mammals, plants or insects and eukaryotes of bacteria, yeasts or cells of mammals, plants or insects.

27) (Amended) A transformed cell expressing the mammalian neuronal amiloride-sensitive proton-activated cationic channels obtained by the method of claim 19.

28) (Amended) A method for screening substances that are capable of modulating activity of mammalian neuronal cationic channels, comprising:

contacting variable quantities of a substance to be tested with the cells according to claim 27;

measuring changes in current caused by the substance on amiloride-sensitive proton-activated cationic channels; and

determining that the substance is capable of modulating activity of the mammalian neuronal cationic channels if the change in current is above a selected value.

29) (Amended) The method according to claim 28, wherein said substance is capable of modulating the perception of acidity and affect nociception and taste transduction.

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**Marked-Up Version to Show Changes Made to the Claims**

1) (Amended) ~~Protein~~An isolated and purified protein constituting a mammalian neuronal cationic ASIC channel that is sensitive to amiloride and activated by protons.

2) (Twice Amended) ~~Protein~~The protein according to claim 1, the amino acid sequence of which is ~~represented in the annexed list of sequences under number~~ SEQ ID NO: 2 or a functionally equivalent derivative ~~of this protein~~.

3) (Twice Amended) ~~Protein~~The protein according to claim 1, the amino acid sequence of which is SEQ ID NO: 4 or a functionally equivalent derivative ~~of this protein~~.

5) (Twice Amended) ~~Protein~~The protein according to claim 1, the amino acid sequence of which is ~~represented in the annexed list of sequences under number~~ SEQ ID NO: 8 or a functionally equivalent derivative ~~of this protein~~.

11) (Twice Amended) ~~Nucleic~~A nucleic acid molecule comprising a nucleic sequence coding for ~~at~~the protein constituting a cationic channel according to one of claims 1, 2, 3 and 5.

12) (Amended) ~~Nucleic~~The nucleic acid molecule according to claim 11, comprising ~~or constituted by~~ the nucleic sequence bounded by nucleotides 123 and 1700 of the sequence represented in ~~the annexed list of sequences under number~~ SEQ ID NO: 1 ~~or its complementary sequence~~.

13) (Twice Amended) ~~Nucleic~~The nucleic acid molecule according to claim 11, comprising ~~or constituted by~~ the nucleic sequence bounded by nucleotides 1 and 1542 of ~~the~~ sequence represented in ~~the annexed list of sequences under number~~ SEQ ID NO: 3 ~~or its complementary sequence~~.

15) (Twice Amended) ~~Nucleic~~The nucleic acid molecule according to claim 11, comprising ~~or constituted by~~ the nucleic sequence bounded by nucleotides 109 and 1785 of

~~the sequence represented in the annexed list of sequences under number SEQ ID NO: 7 or its complementary sequence.~~

17) (Twice Amended) ~~Vector~~A vector comprising at least one nucleic acid molecule according to claim 11, ~~advantageously combined with control sequences.~~

18) (Twice Amended) ~~Method~~A method for producing a protein ~~constituting an ionic channel~~ according to one of claims 1, 2, 3 and 5 comprising ~~the steps of:~~

- transferring a nucleic acid molecule comprising a nucleic acid sequence encoding ~~at~~the protein or a vector comprising said nucleic acid molecule into a cell host,
- culturing said cell host under conditions allowing production of the protein ~~constituting the ionic channel, and~~
- isolating ~~by any suitable means the proteins constituting the ionic channels~~protein.

19) (Twice Amended) ~~Method~~A method for expressing a protein ~~constituting an ionic channel~~ according to one of claims 1, 2, 3 and 5 comprising ~~the steps of:~~

- transferring a nucleic acid molecule comprising a nucleic acid sequence encoding ~~at~~the protein ~~constituting a cationic channel according to one of claims 1, 2, 3 and 5 or a~~ vector comprising said nucleic acid molecule into a cell host, ~~and~~
- culturing said cell host under conditions allowing production of the protein ~~constituting the ionic channel.~~

20) (Twice Amended) ~~Method~~The method according to claim 18, wherein the cell host is a bacteria or a eukaryote cell selected from ~~among the group consisting of prokaryotes or the eukaryotes and notably from among the bacteria, yeasts, or cells of mammals~~mammal cells, plants or insects~~plant cells and insect cells.~~

21) (Twice Amended) ~~Transformed~~A transformed cell expressing the mammalian neuronal amiloride-sensitive proton-activated cationic channels obtained by the method according to claim 18.

22) (Twice Amended) ~~Method~~A method for screening ~~substances that are~~ a substance capable of modulating ~~the~~ activity of mammalian neuronal ~~ionic~~cationic channels, comprising:

contacting variable quantities of a substance to be tested with the cells according to claim 21; ~~and~~

~~measuring, using any suitable means, the effects of said substance on the~~changes in currents of the current caused by the substance on amiloride-sensitive proton-activated cationic channels; ~~and~~

determining that the substance is capable of modulating activity of the mammalian neuronal cationic channels if the change in current is above a selected value.

23) (Twice Amended) ~~Method~~The method according to claim 22, wherein said substance is capable of modulating the perception of acidity; ~~with regard to~~ and affect nociception and taste transduction.

24) (Twice Amended) ~~Pharmaceutical~~A pharmaceutical composition comprising as active ingredient at least one protein ~~constituting an ionic channel~~ according to one of claims 1, 2, 3 and 5 ~~or an antibody directed against said protein.~~

26) (Amended) ~~Method~~The method according to claim 19, wherein the cell host is selected from ~~among the group consisting of prokaryotes or the eukaryotes and notably from among the of~~ bacteria, yeasts or cells of mammals, plants or insects and eukaryotes of bacteria, yeasts or cells of mammals, plants or insects.

27) (Amended) ~~Transformed~~A transformed cell expressing the mammalian neuronal amiloride-sensitive proton-activated cationic channels obtained by the method of claim 19.

28) (Amended) ~~Method~~A method for screening substances that are capable of modulating~~the~~ activity of mammalian neuronal ~~ionic~~cationic channels, comprising:

contacting variable quantities of a substance to be tested with the cells according to claim 27; ~~and~~

~~measuring, using any suitable means, the effects of said substance on the~~changes in currents of the current caused by the substance on amiloride-sensitive proton-activated cationic channels; ~~and~~

determining that the substance is capable of modulating activity of the mammalian neuronal cationic channels if the change in current is above a selected value.

29) (Amended) ~~Method~~The method according to claim 28, wherein said substance is capable of modulating the perception of acidity, ~~with regard to~~and affect nociception and taste transduction.

**Substitute Specification  
(Marked-Up Copy)**



# MAMMAL NEURONAL ACID SENSING CATIONIC CHANNEL, CLONING AND APPLICATIONS THEREOF

## FIELD OF THE INVENTION

The present invention relates to a new family of mammalian, notably human, acidity-  
5 sensitive ionic channels. More particularly, the invention relates to the identification and  
molecular characterization in humans and rats of a new proton-activated cationic channel  
referred to below as "ASIC" to designate the English-language term "Acid Sensing Ionic  
Channel".

## BACKGROUND

10 The ASIC channel constitutes the first member of a group of cationic channels, belonging  
to the family of amiloride-sensitive degenerine sodium channels [6, 11-14], which is  
activated temporarily by extracellular acidification.

Sensitivity to acid is associated with both nociception [1] and the transduction of taste [2].  
The stimulation of sensory neurons by acids is of great importance because acidity  
15 accompanies numerous painful inflammatory and ischemic situations. The pain caused by  
acids is interpreted as being mediated by the cationic channels present at the level of the  
sensory neurons and which are activated by protons [3-5]. The biophysical and  
pharmacological properties of the ASIC channels of the invention are close to those of the  
proton-activated cationic channels described in the sensory neurons [3, 15, 16]. However,  
20 as will be seen in the description below, to date there has been no report of ligand-activated  
ionic channels simpler than the ASIC channels.

## SUMMARY OF THE INVENTION

Thus, the present invention has as its object a protein constituting a neuronal cationic  
channel sensitive to amiloride and activated by protons. More specifically, the invention

relates to the protein constituting the ASIC channel, the amino acid sequence of which is represented in the annexed list of sequences under number SEQ ID NO: 12 or a functionally equivalent derivative of this protein.

Such derivatives are those whose sequence includes a modification and/or a suppression and/or an addition of one or more amino acid residues, as long as this modification and/or suppression and/or addition does not modify the functional and structural properties of the ASIC channel, principally its activation by protons. Such derivatives can be analyzed by the expert in the field using the techniques described in the examples presented below which made it possible to demonstrate the biophysical and pharmacological properties of the ASIC channel.

An example of such a functionally equivalent derivative is the human ASIC protein, the amino acid sequence of which is represented in the annexed list of sequences under number SEQ ID NO: 24, and which is approximately identical to that of the ASIC channel of the rat, designated ASIC1A, represented in the annexed list of sequences under number SEQ ID NO: 1.

Another example of such a functionally equivalent derivative is the protein constituting a cationic channel of degenerine designated "MDEG" [14] or "BNaCl" [20] or designated below as "ASIC2A" or "MDEG1", the amino acid sequence of which is represented in the annexed list of sequences under number SEQ ID NO: 36. MDEG has been described as a mammalian cationic channel sensitive to amiloride which is activated by the mutations responsible for neurodegeneration with the degenerines of *C. elegans*. The MDEG channel is a structural relative of the ASIC channel, the amino acid sequence of which exhibits circa about 67% homology with the ionic channel MDEG. However, the electrophysiological properties of these two channels are different because they are not activated by the same pH changes. Thus, the range of sensitivity of MDEG ( $EC_{50} = 4.05$ ) is different from that of

ASIC ( $EC_{50} = 6.2$ ).

It has been shown that the MDEG channel is activated by the same mutations as those causing neuronal degeneration in *C. elegans*. Thus, like the hyperactive degenerine mutants of *C. elegans*, the active mutants of MDEG are responsible for a-cell death, indicating that the acquisition of function by this neuronal ionic channel could be implicated in various forms of neuronal degeneration of mammals, notably of humans. However, no normal physiological function of MDEG was known until the demonstration of its activation by protons in accordance with the cationic channels of the present invention.

Other examples of proteins constituting a neuronal cationic channel that is sensitive to amiloride and activated by protons according to the invention are presented below:

- A channel designated ASIC1B, whose sequence of 559 amino acids is represented in the annexed list of sequences under number SEQ ID NO: 48. ASIC1B is a splicing variant of the ASIC1A channel cloned from the rat brain by degenerated PCR. The first 185 amino acids are replaced by a new sequence of 218 amino acids which is underlined in SEQ ID NO: 48.

- A channel designated DRASIC, whose sequence of 533 amino acids is represented in the list of sequences under number SEQ ID NO: 510. DRASIC was cloned from sensory neurons from the rat using a partial sequence from the data banks ("Expressed Sequence Tag" with accession number W62694). The properties of DRASIC are as follows:

- It is expressed in the sensory neurons but not in the brain.

- Its expression in *Xenopus* oocytes or in mammal cells allows recording of a proton-activated sodium current which presents two components: a component activating and inactivating itself rapidly and a component activating itself more slowly and not inactivating itself. The two components are selective for  $Na^+$ . A proton-activated cationic channel that does not inactivate itself was implicated in the prolonged sensation of pain caused by acido-



sis.

The invention also relates to a hybrid cationic channel constituted by the combination of a first protein constituting a proton-activated ionic channel according to the invention with a second protein constituting a proton-activated ionic channel. Advantageously, the said  
 5 second protein is also a protein constituting a proton-activated ionic channel according to the invention. As an example of such a combination, one can cite the combination of the protein of the ASIC1A or ASIC2A or DRASIC channel with the protein of the MDEG1 channel, enabling formation of a hybrid channel exhibiting a third range of pH sensitivity (with ASIC:  $EC_{50} = 4.8$ ). Another example of such a hybrid channel is the combination of the protein of  
 10 the ASIC1A, ASIC1B, MDEG1 or DRASIC channels with the protein of the MDEG2 channel.

MDEG2 is a channel that was cloned from the rat brain using a partial mouse sequence accessible in the data banks ("Expressed Sequence Tag with accession number W50528") and whose sequence of 563 amino acids is represented in the annexed list of sequences under  
 15 number SEQ ID NO: 612.

MDEG2 is a splicing variant of MDEG1. The first 185 amino acids are replaced by a new sequence of 236 amino acids which is underlined in SEQ ID NO: 612. MDEG2 is expressed in the brain and in the sensory neurons of the dorsal root ganglia.

MDEG2 expressed alone in *Xenopus* oocytes or in mammal cells does not form a proton-  
 20 activated cationic channel. However, it can combine with MDEG1 or DRASIC to form proton-activated heteromultimeric channels with modified properties:

- The activation pH of the channel formed after the co-expression of MDEG1 and MDEG2 is different. After expression in COS cells, the current has not reached its maximum value at pH 3 whereas the current induced by MDEG1 alone is saturated at a pH between 4.5 and

4.0.

- The inactivation kinetics and the ionic selectivity of the channel formed after the co-expression of MDEG1 and MDEG2 are clearly different from those of MDEG1 alone. A current appears which inactivates itself slowly and is barely selective for Na<sup>+</sup> and K<sup>+</sup>.

- 5    - The sodium current obtained after expression of DRASIC becomes nonselective (it does not differentiate between sodium and potassium) when MDEG2 is co-expressed with DRASIC. This new property is similar to that of the proton-activated cationic channel which is implicated in the prolonged sensation of pain caused by acidosis. It is very probable that DRASIC and MDEG2 are part of this channel.

- 10    The amino acid sequence homologies of the proteins constituting the ASIC1A, ASIC1B channels cited according to the invention are presented in ~~table~~ Table 1 below.

Table 1

Channel	ASIC 1B	ASIC 1A	MDEG2	MDEG1	DRASIC
15 ASIC1B	100	80	56	61	52
ASIC1A		100	59	68	53
MDEG2			100	78	48
MDEG1				100	51
DRASIC					100

20

Polyclonal or monoclonal antibodies directed against at least one protein constituting an ionic channel of the invention and/or against a hybrid channel as above can be prepared by the classic methods described in the literature. The antibodies are useful for investigating the presence of the ionic channels of the invention in various human and animal tissues, but

25    due to their specificity they can also find applications in the therapeutic domain for inhibiting or activating *in vivo* an ASIC channel and/or its derivatives.

The present invention also has as its object a nucleic acid molecule comprising or constituted by a nucleic sequence coding for a protein constituting a neuronal cationic channel that is sensitive to amiloride and activated by protons. More particularly, the invention relates to a nucleic acid molecule comprising at least one sequence coding for the protein constituting the ASIC channel whose amino acid sequence is represented in the annexed list of sequences under number SEQ ID NO: 12 or for a functionally equivalent derivative of this protein. A DNA molecule comprising the sequence coding for the ASIC protein is the one represented in the annexed list of sequences under number SEQ ID NO: 1 or its complementary sequence. Another DNA molecule according to the invention is the one represented in the annexed list of sequences under number SEQ ID NO: 23 or under number SEQ ID NO: 35 or their complementary sequence.

A DNA molecule comprising the sequence coding for the ASIC1B protein is that of 3647 ~~pbbp~~ represented in the annexed list of sequences under number SEQ ID NO: 47 or its complementary sequence. More specifically, the invention relates to the nucleic sequence comprised between nucleotides 109 and 1785 of the sequence represented in the annexed list of sequences under number SEQ ID NO: 47 or its complementary sequence.

A DNA molecule coding for the DRASIC protein is that of 1602 ~~pbbp~~ represented in the annexed list of sequences under number SEQ ID NO: 59 or its complementary sequence.

A DNA molecule coding for the ~~MDEG1~~MDEG2 protein is that of 1602 ~~pbbp~~ represented in the annexed list of sequences under number SEQ ID NO: 611 or its complementary sequence.

The invention also relates to a vector comprising at least one of the preceding nucleic acid molecules, advantageously combined with suitable control sequences, as well as a procedure for production or expression in a cell host of a protein constituting an ionic channel according to the invention. The preparation of these vectors as well as production or

expression in a host of the channels of the invention can be implemented by means of the molecular biology and genetic engineering techniques known to the expert in the field.

As an example, a production method for a protein constituting a cationic channel according to the invention is comprised of:

- 5    – transferring a nucleic acid molecule according to the invention or a vector containing said molecule into a cell host,
- culturing said cell host under conditions allowing production of the protein constituting the cationic channel,
- isolating by any suitable means the proteins constituting the ionic channels of the
- 10 invention.

As an example, a method for expressing an ionic channel according to the invention is comprised of:

- transferring a nucleic acid molecule of the invention or a vector containing said molecule into a cell,
- 15    – culturing said cell host under conditions allowing expression of the ionic channels of the invention.

The cell host employed in the preceding methods can be selected from among the prokaryotes or the eukaryotes and notably from among the bacteria, yeasts or cells of mammals, plants or insects.

- 20    The vector used is selected in relation to the host to which it will be transferred; any vector such as a plasmid can be used.

The invention thus also relates to the transformed cells expressing ASIC channels and/or its derivatives such as MDEG obtained according to the preceding methods. These cells are useful in screening for substances capable of modulating the perception of acidity with

regard to both nociception and taste transduction. This screening is implemented by bringing variable quantities of a substance to be tested into contact with cells expressing the ASIC channels then measuring, by any suitable means, the possible effects of said substance on the currents of said channels. Electrophysiological techniques also enable these studies and are  
5 also the object of the present invention when employing ASIC channels or their derivatives. These screenings allow identification of new drugs that are useful in the treatment or prevention of pain. They also enable investigation of agents that modulate acid taste. In addition, they enable investigation of blockers that can inhibit neurodegenerations induced by hyperexpression of these channels. These drugs which are isolated and detected by means  
10 of the methods above are also part of the invention. The ASIC channels clearly have ionic selectivity properties, notably with regard to their selective permeability by sodium, potassium and calcium, which endows them with excitotoxic properties when hyperstimulated.

A protein constituting an ASIC neuronal ionic channel can also be useful for the  
15 fabrication of drugs intended for the treatment or prevention of pathologies entailing the painful perception of acidity which intervenes in inflammatory diseases, ischemias and a certain number of tumors. The invention thus also relates to the pharmaceutical compositions comprising as active ingredient at least one protein constituting an ionic channel according to the invention.

20 A nucleic acid molecule coding for a protein constituting an ASIC channel or a derivative thereof, or a vector comprising this nucleic acid molecule or a cell expressing ASIC channels are also useful for the preparation of transgenic animals. These can be animals superexpressing said channels, but especially so-called "knock-out" animals, i.e., animals ~~presenting with~~ having a deficiency in these channels; these transgenic animals are prepared  
25 by methods known to the expert in the field, and enable production of live models for

studying animal pathologies associated with the ASIC channels.

The nucleic acid molecules of the invention or the cells transformed by said molecule can thus be used in genetic therapy strategies in order to compensate for a deficiency in the ASIC channels at the level of one or more tissues of a patient. The invention thus relates also to  
5 a drug comprising nucleic acid molecules of the invention or cells transformed by said molecules for the treatment of pathology involving the ASIC channels or their derivatives.

In addition to the property of being activated by protons and the resultant applications described above in the domain of the perception of acidity, the ASIC channel, because of its structural relationship with the MDEG channel, can act like a neuronal degenerine following  
10 mutation.

The death of certain neurons is characteristic of many types of neuronal degenerative disorders such as Alzheimer's disease, Huntington's disease, Parkinson's disease, amyotrophic lateral sclerosis and cerebellar ataxia. Only a few deficient genes are known and many more remain to be identified. The primitive neural network of the nematode *C. elegans* constitutes a good model of neuronal development and death. The hereditary  
15 degeneration in *C. elegans* can be due to mutations of the degenerines deg-1, mec-4 and mec-10. The homologies with the subunits of the amiloride-sensitive sodium channel, the functional expression product of the mec-4 chimeras of the epithelial sodium channel, suggest that the degenerines are ionic channels whose acquisition of function is the cause of  
20 neuronal degeneration.

The present invention thus also relates to application of the ASIC channel for studying these pathological modifications that lead to degenerations. The techniques employed for these applications, for example for drug screening, are similar to those described above for the investigation of taste-modulating agents and analgesic agents.

In addition, a protein constituting an ASIC neuronal ionic channel, an agonist or an antagonist of said protein, can also be used for the fabrication of drugs intended for the treatment or prevention of pathologies involving cerebral neuronal degeneration. The invention thus also relates to the pharmaceutical preparations comprising as active ingredient  
 5 at least one of these proteins possibly combined with a physiologically acceptable vehicle.

More specifically, the invention relates to a chemical or biological substance that is capable of modifying the currents of an ionic channel and/or a hybrid channel according to the invention for the preparation of a drug capable of modulating the perception of acidity with regard to nociception as well as taste transduction in a human or animal subject.

## 10 BRIEF DESCRIPTION OF THE SEQUENCES AND DRAWINGS

Other characteristics and advantages of the invention will be seen in the description below related to research activities that led to the demonstration and the characterization of the ASIC channel, and in which reference will be made to the annexed sequences and drawings in which:

- 15    - SEQ ID NO: 1 represents the sequence of 526 amino acids of the protein of the ASIC channel deduced from the cDNA sequence of the rat (SEQ ID NO: 1).
- SEQ ID NO: 2 represents the partial sequence of 514 amino acids of the protein of the ASIC channel deduced from the partial sequence of human cDNA (SEQ ID NO: 3).
- SEQ ID NO: 3 represents the sequence of 512 amino acids of the protein of the MDEG  
 20 channel deduced from the sequence of human cDNA (SEQ ID NO: 5).
- SEQ ID NO: 4 represents the sequence of 559 amino acids of the protein of the ASIC1B channel as well as the sequence of a DNA molecule comprising the sequence coding for that protein (SEQ ID NO: 7).
- SEQ ID NO: 5 represents the sequence of 533 amino acids of the protein of the

DRASIC channel and the sequence of DNA coding for that protein (SEQ ID NO: 9).

- SEQ ID NO: 612 represents the sequence of 563 amino acids of the protein of the MDEG2 channel as well as the sequence of a DNA molecule comprising the sequence coding for that protein (SEQ ID NO: 11).

- 5 - ~~Figure~~Fig. 1 shows a sequence which represents the alignment of the sequences of the rat ASIC proteins (at top) and human ASIC proteins (at bottom) of sequences SEQ ID NO: 12 and SEQ ID NO: 24. Comparison of these sequences shows the absence of 14 amino acids at the beginning of the human coding phase compared to that of the rat.

~~Figure~~Fig. 2 ~~represents a comparison~~represent comparisons of the protein sequence of the  
10 ASIC (SEQ ID NO: 2) channel with the ~~sequence~~sequences of other ionic channels:

A - MDEG (SEQ ID NO: 6) [14], a mammalian cationic channel that is activated by the mutations responsible for neurodegenerations with the degenerines of *C. elegans*.

B - FaNaCh (SEQ ID NO: 13) [10], a peptide of a sodium channel of *Helix aspersa* that is activated by FMRFamide.

- 15 C - The degenerine MEC-4 (SEQ ID NO: 14) [12] of *C. elegans*.

~~In this figure, the~~The residues that are identical or similar to those of ASIC are printed respectively in white on a black background and in black on a gray background. The supposed transmembranal regions (MI, MII) of ASIC are marked by black bars.

~~Figure~~Fig. 3 ~~represents~~shows the phylogenetic tree of the proteins of the subunits  $\alpha$ NaCh,  $\beta$ NaCh,  $\gamma$ NaCh,  $\delta$ NaCh of the amiloride-sensitive sodium channel and of the degenerines MEC-4, MEC-10 and DEG-1 of *C. elegans*.  
20

~~Figure~~Fig. 4 ~~represents~~shows the topology proposed for this latter family of ionic channels [30].

~~Figure~~Fig. 5 shows graphs of the biophysical properties of the proton-activated ASIC1  
25 channel.



- In a: the macroscopic inflowing currents recorded at -70 mV after rapid pH changes from pH 7.4 to pH 6.
  - In b: The dose-response curve of the extracellular pH. The initial pH was 7.4 and the points represent the mean values from 6 tests. The insert in this figure shows the typical responses at -70 mV.
  - In c: the Q-V relations of the outside-out patch with 140 mM of Na<sup>+</sup> (■) or of Li<sup>+</sup> (●) in the bath solution. Q is the charge transported during the acid pH transition. The insert in this figure shows the typical responses in a medium containing Na<sup>+</sup>.
  - In d: the currents activated by the H<sup>+</sup> protons recorded at various potentials in an outside-out patch in a medium containing Na<sup>+</sup>.
  - In e: the mean i-V relations measured from the outside-out patch with 140 mM of Na<sup>+</sup> (■), 140 mM of Li<sup>+</sup> (●) or 1.8 mM of Ca<sup>2+</sup> (▲), as majority permeable ions in the external solutions; the inversion potentials were respectively 65 mV, 58 mV and -34 mV.
  - In f: the proton current through the ASIC1 channel. The relations between the current peak and the voltage were measured from an outside-out patch in a solution of free Na<sup>+</sup>, free Ca<sup>2+</sup> with pipettes containing a solution of free K<sup>+</sup>, at pH 4 (●) and at pH 3 (■), with (▲) representing the results obtained under the same conditions as (■) but with KCl in the pipette. The insert in this figure shows the typical responses under (▲) conditions.
- Figure** Fig. 6 shows graphs of the effect of Ca<sup>2+</sup> and of amiloride on the ASIC current.
- In a: the currents activated by the H<sup>+</sup> protons recorded at various membranal potentials from an outside-out patch with 1.8 mM of Ca<sup>2+</sup> in a solution of free Na<sup>+</sup>; the currents were inverted at -35 mV.
  - In b: the mean Q-V relations from an outside-out patch recorded in solutions of free Na<sup>+</sup> containing 1.8 mM of Ca<sup>2+</sup> (○, inversion potential -34 mV) or 0.1 mM of Ca<sup>2+</sup> (●, inversion potential -80 mV).

- In c: the effect of the external  $\text{Ca}^{2+}$  on the macroscopic peak of inflowing current recorded at -70 mV and activated by a rapid pH change from pH 7.4 to pH 6. The insert in this figure shows the typical responses. The points represent means values  $\pm$  se of 5 oocytes.

- In d: the effect of amiloride on the currents activated by the  $\text{H}^+$  protons recorded at 0 mV  
5 from an outside-out patch.

- In e: the inhibition of the macroscopic current (induced by a pH change from pH 7.4 to pH 6) at -70 mV by amiloride and derivatives. The points represent the means values  $\pm$  se of 5 oocytes.

**Figure** Fig. 7 shows the tissue distribution of ASIC channel mRNA.

10 - In a: Northern blot analysis of the mRNA expression of the ASIC channel in human tissues.

- In b: RT-PCR analysis of the mRNA expression of the ASIC channel in the rat brain and in the dorsal root ganglion (DRG). (+), (-) represent respectively the samples with or without reverse transcriptase. The agarose gel sections were developed in 1% ethidium  
15 bromide. The arrows indicate the discounted size (657 pb) of the PCR product.

**Figure** Fig. 8 ~~represents the~~ shows photographs of *in situ* hybridization.

- In a and b: hybridization of 6- $\mu\text{m}$  sections of a dorsal root ganglion from a 3-year-old rat with the E probe marked with digoxigenin. In a: a low-lighting microphotograph (enlargement 30 X). In b: a high-resolution image (enlargement 80 X) of a: One can see  
20 the intense marking of the small-diameter neurons (arrows). Similar results were also obtained with probes A, C and D.

- In c: the distribution of the ASIC channel mRNA in the brain of an adult rat analyzed by in-situ hybridization with antisense oligonucleotide C. Identical results were obtained with oligonucleotide B. The colors indicate abundance (red: high expression; blue: not  
25 detectable). The abbreviations used in the figure are as follows: Cer = cerebellum; Hip =

hippocampus; OB = olfactory bulb; Cx = cortex.

## DETAILED DESCRIPTION

### I - Materials and Methods

#### 5 1) Cloning the ASIC channel.

The conserved sequences of the family of ASIC ionic channels were used to prepare the following PCR primer sequences:

TTYCCIGCIRTIACIITNTGYAAY (SEQ ID NO: 15), and

CAIARICCIAIITGNCCNCCDAWRTC (SEQ ID NO: 16).

- 10 A bank of rat brain cDNA (Stratagène #936515) was hybridized with the PCR product of 1 kB of rat brain and the partial clones were isolated. The fifth extremity of the cDNA (202 bp) was isolated by PCR after ligation adapted to the double-strand cDNA.

#### 2) Electrophysiology

- 0.25 ng of cRNA was injected into the *Xenopus laevis* oocytes and the recording  
15 microelectrodes for the imposed voltage and for the patch-clamp were installed two days after the injection. The bath solutions for the outside-out patch recordings and the pipettes for the outside-out patch and total cells recordings contained: 140 mM KCl (or NMDG), 2 mM MgCl<sub>2</sub>, 5 mM EGTA, 10 mM Hepes, pH 7.4 (with KOH). The pipettes for the outside-out patch recordings and the bath solutions for the outside-out patch and total cells  
20 recordings contained: 140 mM NaCl (or LiCl or NMDGCl), 2 mM MgCl<sub>2</sub>, 1.8 mM CaCl<sub>2</sub>, 10 mM Hepes, pH 7.4 (adjusted with HCl, NaOH, LiOH or TMAOH). The rapid pH changes from the initial pH were obtained by perfusion with a bath solution adjusted to the pH indicated in the figures. The intracellular acidification of the oocytes was implemented

by injecting 50 ml of the internal solution at pH 2 or by perfusion and withdrawal of a bath medium containing 20 mM  $\text{NH}_4\text{Cl}$ . None of the recorded currents was contaminated by the  $\text{Ca}^{2+}$  current sensitive to the  $\text{Cl}^-$  of the *Xenopus* oocyte. The data were sampled at 2 kHz and filtered at 500 Hz for the analysis (Logiciel Biopatch).

### 5    3) Northern blot analysis, RT-PCR and *in-situ* hybridization

The Northern blot kit was obtained from Clontech Co. (Palo Alto, CA) and contained circaabout 2  $\mu\text{g}$  of poly(A<sup>+</sup>) RNA per line. The blot was hybridized with a fragment of the partial human clone (corresponding to bases 270 to 764 of the rat clone) marked with  $^{32}\text{P}$  at 65°C in 6xSSC. For the RT-PCR analysis, 5  $\mu\text{g}$  of rat brain total RNA and 3  $\mu\text{g}$  of dorsal  
10 root ganglion were reverse transcribed and 1/30 of the sample was amplified by 30 PCR cycles with the following sequence primers:

ATTGCTCTTCCCATCTCTAT (SEQ ID NO: 17), and

TTCAAGGCCCATACCTAAGT (SEQ ID NO: 18).

The negative controls were treated in an identical manner with the exception of the reverse  
15 transcriptase which was not added. The antisense oligonucleotides corresponding to base 70 to 114 (A), 215 to 248 (B), 1821 to 1859 (C), 1896 to 1940 (D) and the double-strand DNA corresponding to base 1685 to 2672 were used for the *in-situ* hybridizations. The sections of adult rat brain were hybridized with oligonucleotides B or C the ends of which were marked with  $^{32}\text{P}$  for one night at 37°C in 50% formamide, 2xSSC, then washed at am-  
20 bient temperature in 1xSSC. The signal was eliminated by 500-times excess of unmarked oligonucleotides. The dorsal root ganglion sections were hybridized with oligonucleotides A, C or D marked with digoxigenin (DIG)-dUTP and with probe E marked with DIG-dUTP by PCR. The marking of the probes, the preparation of the samples, the hybridization and the visualization of the DIG nucleic acids with alkaline phosphatase conjugated with anti-

DIG antibodies were performed in accordance with the supplier's protocols (Boehringer Mannheim).

#### 4) Computer analysis

The sequence alignments and the phylogenetic tree (Kimura substitution, UPGMA option) were performed with the GCG program (Genetics Computer Group, Madison, WI).

## II – Results

The 35 kb cDNA isolated from rat brain codes for a protein of 526 amino acids that exhibits, as shown in ~~figure~~Fig. 2, homologies with all of the cloned members of the family of amiloride-sensitive degenerative sodium channels.

As shown in ~~figure~~Fig. 5, expression of the cRNA in the *Xenopus* oocytes induced an inflowing current activated by H<sup>+</sup> protons. The biophysical and pharmacological properties of the ASIC channel are close to those described for the proton-activated cationic channels of sensory neurons [3, 15, 16]. Reduction of the extracellular pH below a pH of 6.9 activates a rapidly rising and desensitized inflowing current (~~figure~~Fig. 5a and b). This channel is activated by extracellular protons since, as shown in ~~figure~~Fig. 5 (c and d), application of an acid on the extracellular surface of the outside-out patch activates the channel. Intracellular acidification of oocytes and acidification of the intracellular surface of the outside-out patch does not activate the ASIC channel nor alter the ASIC current induced by the extracellular protons.

The analysis of curves I-V of ~~figure~~Fig. 5 (c and e) recorded with different extracellular cations shows that Na<sup>+</sup> is the majority permeable ion (simple conductance channel 14.3 pS). Like the proton-sensitive ionic channel of the sensory neurons [15, 16], the ASIC channel discriminates weakly between the cations (~~figure~~Fig. 5c, e, f). In fact, the channel is also permeable to Li<sup>+</sup>, K<sup>+</sup>, Ca<sup>2+</sup> and H<sup>+</sup> with the ratios  $pNa^+/pLi^+ = 1.3$  (~~figure~~Fig. 5c, e),  $pNa^+/pK^+$

=13 (figureFig. 5c, e),  $pNa^+/Ca^{2+} = 2.5$  (figureFig. 5e) and  $pNa^+/H^+ = 0.8$  (figureFig. 5f). The permeability to  $Ca^{2+}$  of ASIC could be a voltage-independent entry path of  $Ca^{2+}$  into the cell. An inflowing current of  $Ca^{2+}$  into the cell via the ASIC channels can be detected in the absence of extracellular  $Na^+$  (figureFig. 6a, b). As indicated in figureFig. 5 (e), the unitary  
 5 conductance for  $Ca^{2+}$  was 5.2 pS. In the presence of 140 mM of extracellular  $Na^+$ , augmentation of the concentrations of external  $Ca^{2+}$  diminished the amplitude of the current activated by the protons (FigureFig. 6c), thereby demonstrating that  $Ca^{2+}$  inhibits the permeability to  $Na^+$ . Blockage by external  $Ca^{2+}$  is characteristic of the  $I(H^+)$  of the sensory neurons [17]. The inflowing current activated by  $H^+$  in the sensory neurons is inhibited by  
 10 amiloride [18] and ethylisopropylamiloride (EIPA) [19]. As shown in figureFig. 6 (d, e), the ASIC channel exhibits the same pharmacology and is blocked in a reversible manner ( $K_d = 10 \mu M$ ) by amiloride and its derivatives benzamil and EIPA.

In addition, the ASIC channel protein exhibits approximately 67% sequence homology with the degenerine ionic channel referred to as "MDEG" [14] or "BNaCl" [20]. However,  
 15 the electrophysiological properties of these two clones expressed in *Xenopus* oocytes are clearly different:

- As shown in figureFig. 5a, the MDEG channel is not activated by the same pH changes as the ASIC channel.
- Substitution of the glycine residue in position 430 of MDEG by an acid-inhibiting amino  
 20 acid such as valine or phenylalanine activates the channel [14], just as the mutation of alanine in position 704 of degenerine MEC-4 causes neurodegeneration in *C. elegans* [12]. Identical mutations of ASIC (glycine in position 431 replaced by valine or phenylalanine) do not lead to activity and the mutants can not be activated by protons.

Proton-activated cationic channels have been described not only in the sensory neurons but  
 25 also in the neurons of the central nervous system [21]. The tissue distribution of the

expression of the mRNA of the ASIC channel is in agreement with this observation. As shown in ~~figure~~Fig. 7a, a 4.3-kb transcript was detected in the brain by Northern blot analysis and the PT-PCR results presented in ~~figure~~Fig. 7b show that the dorsal root ganglion expresses the ASIC mRNA. ~~Figure~~Fig. 8 (a, b) shows that ASIC mRNA is well expressed  
 5 by the small neurons of the dorsal root ganglion, which supports the fact that ASIC is the rapidly desensitizing proton-activated cationic channel described in the nociceptive sensory neurons. Whereas the presence of proton-activated cationic channels in the dorsal root ganglion is in agreement with their function of acidity detector in nociception, their role in the brain remains to be established. The results of *in-situ* hybridization in ~~figure~~Fig. 8c show  
 10 a broad and heterogeneous expression of the ASIC channel mRNA. The highest levels of expression were observed in the principal olfactory bulb, the cerebral cortex, the hippocampus, the habenula, the basolateral amygdaloid nucleus and the cerebellum. The synaptic activity accompanies extracellular pH changes [22, 23] and the rapid localized pH changes in or close to the synaptic cleft are noticeably more saturated and stronger than the  
 15 reported macroscopic fluctuations in the pH.

The proton-activated cationic channels are the only known ionic channels that are directly activated by a change in pH and it was envisaged that the extracellular fluctuations in pH played a neuromodulator role [23]. The expression of cationic channels in the brain supports in addition the hypothesis that the pH fluctuations are not solely a neuronal activation by a  
 20 product, but even more a communications pathway in the central nervous system.

In addition to the rapidly inactivated proton-activated cationic channels, the presence has been reported in the sensory neurons of proton-activated cationic channels exhibiting slower kinetics [4, 24]. The proton-activated cationic channels probably form, like other cationic channels activated by a ligand [25, 26], a family of cationic channels in which different  
 25 subunits or combinations of subunits constitute channels with diverse pharmacological and

biophysical properties.

The sensation of acidity is not uniquely implicated in nociception but is also associated with the transduction of taste [2]. Acid stimulations activate the proton-activated cationic channels in the taste cells [2, 27] and amiloride inhibits the perception of acid taste [2]. Also, the physiological as well as pharmacological data indicate that ASIC and other member of this family are implicated in the transduction of taste. It is, in fact, especially surprising that the same class of ionic channels is associated with different facets of sensory perception:

- The amiloride-sensitive sodium channels are associated with the transduction of salty taste [2].

- 10    - The degenerines of *C. elegans* are implicated in mechanotransduction and have been proposed as forming the mechanosensitive ionic channels [28, 29].

- The ASIC channels are implicated in nociception and the transduction of acid taste.

Cloning of the ASIC channel provides a new tool for the investigation of this group of ionic channels and the development of specific blockers for use notably as analgesic agents.



Pages 22-23 = List of references

Pages 24-43 = LIST OF SEQUENCES

NUMBER OF SEQUENCES: 6

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INFORMATION CONCERNING SEQ ID NO: 1

- i) CHARACTERISTIC OF THE SEQUENCE:
  - A) LENGTH: 3562 base pairs
  - B) TYPE: nucleic acid
  - C) NUMBER OF STRANDS: double
  - D) CONFIGURATION: linear
- ii) TYPE OF MOLECULE: DNA
- vi) ORIGIN: rat
- ix) CHARACTERISTIC
  - A) NAME/KEY: ASIC
  - B) LOCALIZATION: 123 .. 1700
- xi) DESCRIPTION OF THE SEQUENCE: SEQ ID NO: 1:

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INFORMATION CONCERNING SEQ ID NO: 2

- i) CHARACTERISTIC OF THE SEQUENCE:
  - A) LENGTH: 1620 base pairs
  - B) TYPE: nucleic acid
  - C) NUMBER OF STRANDS: double
  - D) CONFIGURATION: linear
- ii) TYPE OF MOLECULE: DNA
- vi) ORIGIN: human
- ix) CHARACTERISTIC
  - A) NAME/KEY: ASIC
  - B) LOCALIZATION: 1 .. 1542
- xi) DESCRIPTION OF THE SEQUENCE: SEQ ID NO: 2:

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INFORMATION CONCERNING SEQ ID NO: 3

- i) CHARACTERISTIC OF THE SEQUENCE:
  - A) LENGTH: 1666 base pairs
  - B) TYPE: nucleic acid
  - C) NUMBER OF STRANDS: double
  - D) CONFIGURATION: linear
- ii) TYPE OF MOLECULE: DNA
- vi) ORIGIN: human
- ix) CHARACTERISTIC
  - A) NAME/KEY: MDEG
  - B) LOCALIZATION: 127 .. 1663
- xi) DESCRIPTION OF THE SEQUENCE: SEQ ID NO: 3:

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<u>INFORMATION CONCERNING SEQ ID NO: 4</u>	
i)	CHARACTERISTIC OF THE SEQUENCE:
A)	LENGTH: 3647 base pairs
B)	TYPE: nucleic acid
C)	NUMBER OF STRANDS: double
D)	CONFIGURATION: linear
ii)	TYPE OF MOLECULE: DNA
vi)	ORIGIN: rat
ix)	CHARACTERISTIC
A)	NAME/KEY: ASIC1B
B)	LOCALIZATION: 109 .. 1785
xi)	DESCRIPTION OF THE SEQUENCE: SEQ ID NO: 4:

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<u>INFORMATION CONCERNING SEQ ID NO: 5</u>	
i)	CHARACTERISTIC OF THE SEQUENCE:
A)	LENGTH: 1602 base pairs
B)	TYPE: nucleic acid
C)	NUMBER OF STRANDS: double
D)	CONFIGURATION: linear
ii)	TYPE OF MOLECULE: DNA
vi)	ORIGIN: rat
ix)	CHARACTERISTIC
A)	NAME/KEY: DRASIC
B)	LOCALIZATION: 1 .. 1602
xi)	DESCRIPTION OF THE SEQUENCE: SEQ ID NO: 5:

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INFORMATION CONCERNING SEQ ID NO: 6

- i) CHARACTERISTIC OF THE SEQUENCE:
  - A) LENGTH: 1948 base pairs
  - B) TYPE: nucleic acid
  - C) NUMBER OF STRANDS: double
  - D) CONFIGURATION: linear
- ii) TYPE OF MOLECULE: DNA
- vi) ORIGIN: rat
- ix) CHARACTERISTIC
  - A) NAME/KEY: MDEG2
  - B) LOCALIZATION: 16 .. 1707
- xi) DESCRIPTION OF THE SEQUENCE: SEQ ID NO: 6:

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